THE INFLUENCE OF TEMPERATURE ON OSMOTIC REGULATION IN TWO SPECIES OF ESTUARINE SHRIMPS (PENAEUS)

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Estuarine decapod Crustacea living in an environment of varying salinities and temperatures face great osmoregulatory problems. Presumably such animals could either possess well-developed osmoregulatory powers or, lacking these, follow passively the fluctuations of the external medium. Migratory crustaceans present a special case, for their movements may carry them in and out of estuaries, often quite rapidly, thus complicating their osmoregulatory work.

Among swimming shrimps (Penaeidae) are many which live a wandering life, and on the southeastern and Gulf coasts of the United States three such species, *Penaeus setiferus* (Linn.), *P. duorarum* Burkenroad and *P. aztecus* Ives, occur in enormous numbers in and near estuaries.

The geographic ranges, life histories, migratory habits and general ecology of these species have been reported in whole or in part in numerous publications, including: Burkenroad (1934, 1939); Gunter (1950); Heegaard (1953); Lindner and Anderson (1956); Pearson (1939) and Williams (1955). It is well established that these species spawn at sea. The demersal eggs hatch into nauplii which undergo larval transformations while being transported shoreward; by the time they are transformed to the postlarval stage, they are entering estuaries where they proceed to shallow brackish areas to continue growth. With increasing age they move into deeper estuarine waters, return to sea with approaching sexual maturity, and seldom, if ever, return to the estuaries. The estuarine phase lasts about four months in summer but is extended in colder months.

Knowing this, the questions asked in this study are: do these animals regulate their internal salinities and, if so, how well do they accomplish osmoregulation in a variety of salinity and temperature combinations?

Similar questions have been posed by others. Panikkar (1941) found that *Leander serratus, L. squilla* and *Palaemonetes varians* in undiluted sea water are able to maintain their internal fluids at lower concentrations than the outside medium (hypotonic regulation), and in waters of low-salt content are able to maintain their internal fluids at higher concentrations than the outside medium (hypertonic regulation). Panikkar and Viswanathan (1948) found the same ability exhibited by *Metapenaeus monoceros* and Panikkar (1951) reported it shown to a lesser extent in *M. dobsoni, Penaeus indicus* and *P. carinatus* and in the grapsoid crabs *Eriocheir sinensis* and *Varuna litterata*. Panikkar (1951: page 171) states that this ability to regulate is "probably the most elaborate osmotic mechanism ever perfected by invertebrates" and is widely distributed among the

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Crustacea. Broekema (1941) and Verwey (1957) have shown that the blood of *Crangon crangon* is hypotonic in sea water and hypertonic in diluted sea water. Similar osmoregulatory patterns in a number of grapsoid and ocypodid crabs have been found by Edmonds (1935), Jones (1941), Gross (1955, 1957a) and in *Birgus latro* by Gross (1955).

The relationship of osmoregulation to temperature has not been so extensively investigated. Broekema (1941) found in *Crangon crangon* that young in summer tolerate lower salinities than adults. Adults seek higher salinities in cold weather and lower salinities in warm weather. The young, after a period of estuarine existence, go to sea in the fall and return to estuaries as adults the following summer, but they never again inhabit waters of as low salinity as they experienced when young. Broekema found that continued dilution of the external medium brought about continued dilution of the internal medium within limits, but in a constant salinity the concentration of blood increased with a fall in temperature.

Materials and Methods

Though three species of *Penaeus* are abundant in North Carolina, only two, *duorarum* and *aztecus,* are available in large numbers in nursery areas near Morehead City each year. These two species were used in this study. From spring to late summer *aztecus* is abundant on the nursery grounds and from midsummer to fall and winter *duorarum* is available. Because a wide range of sizes is represented in both species during the year and because shrimp of different sizes occupy different habitats, each species was divided arbitrarily into two groups, juveniles (under 100 mm. total length) and subadults (100 mm. and over). Only *aztecus* attained the larger sizes during the experimental period.

The problem of osmoregulation in these penaeids was approached as simply as possible. Freezing point depression (melting point) of blood was used as an indication of the ability of these animals to osmoregulate in various dilutions of sea water at different temperatures. Using modifications of the methods of Jones (1941), Gross (1954) and Hickman (1959) for determining melting points, small, uniform samples (approximately 1 $mm.^3$) of blood were taken in dried, thin-walled capillary tubes (1 mm. diameter), the ends of the tubes sealed with Vaseline, and the samples quick-frozen on dry ice. Samples were stored in a freezer until the melting points could be determined. Melting points of known standards of pure and salt water were determined with the aid of a differential thermometer.

Later, determinations of the unknowns were made by timing the end points of melting of the frozen crystals in the unknowns and standards as they warmed slowly in a waterproof, insulated box filled with ice brine, and by converting the melting times to degrees C.

Samples of blood were taken from individual shrimp by puncturing the sternum of the first abdominal segment with a pin or sharp awl, then catching the fluid in a capillary tube. Prior to making the puncture, the sternum and adjacent structures were thoroughly dried with absorbent cotton swabs. In large individuals, 70 mm. and above, fluid welled out and entered easily into the tubes by capillarity. In younger individuals the amount of fluid was often so small that it had to be sucked into drawn capillary tubes with the aid of a vaccine bulb. Most shrimp

stood this treatment well (though a few small individuals were lost) and the wounds closed readily.

Experimentation was limited to juvenile and subadult shrimp, and experimental conditions for salinity and temperature approximated those found in the sounds seasonally, where salinity ranges from about 7‰ to 34‰ and temperature from 4° to 30° C.

Five series of round, wide-mouthed, five-gallon jars were used as aquaria. Each series consisted of five jars containing salinities of approximately 10, 15, 20, 25 and 30‰ sea water (hydrometer readings). Dilutions were made with tap water. Water was aerated for 24 hours at room temperature before animals were introduced. At the beginning of an experiment shrimp were transferred from outside holding tanks supplied by a running sea water system to the jars, four individuals per jar. (The system pumps 30–34‰ Sound water through hard rubber and polyethylene pipes.) Shrimp in almost all cases were caught a day or two before each experiment began. Prior to each experiment samples of water and blood from shrimp were taken from the outside holding tanks. One blood sample was taken at random from shrimp in each jar after shrimp were placed in jars for two to three hours at room temperature (25–32° C.). Shrimp sampled were returned to the jars. (The few specimens badly injured or killed in the initial sampling were discarded and replaced.) At this same time a water sample in a capillary tube was taken from each jar as a check on the salinity mixtures. After samples were taken, four series were subjected to gradually lowered temperatures in a cold room. The fifth series was maintained as a control at room temperature throughout the experimental period. In 48 hours the temperature was lowered from about 28° to 18° C. and another round of blood samples was taken; in 96 hours at 8° C. another round was taken. At the end of 96 hours all animals were discarded, the water thrown out, new mixtures prepared, and fresh shrimp used for replications. Animals were not fed during the experimental periods.

A number of difficulties were experienced with the technique described above. It is desirable to have the brine solution warm slowly, one degree C. per half hour (Gross, personal communication). I had trouble maintaining this rate of warming, even when the box was precooled, for on hot humid days (temperature near 32° C. and humidity near saturation) the box would sometimes warm at a rate of one degree in fifteen minutes. Securing samples of uniform volume in the initial round of samples each week in this climate in summer was difficult. At temperatures above 22° C. shrimp blood coagulates rapidly, almost on contact with glass. The initial blood samples were taken from animals in air temperatures of 27°–32° C. and extreme difficulty was experienced in securing unclotted and uniform samples. As a corrective measure initial sampling was done in the cold room which had been quickly cooled to air temperature of 18° to 22° C. (water in the aquaria remained at approximately 28° C.). This method helped but still the amount of clotting hindered taking samples of uniform volume. This lack of uniformity in sample size gave somewhat distorted results in terms of melting points, but replications smoothed the inequalities. The 48- and 96-hour samples were readily taken at the lower temperatures.

For the experimental series, replications of individual determinations for both blood and water were averaged (Table I). The average values were subjected to

standard methods for linear regression analysis (Snedecor, 1956) in which melting point of blood was plotted against melting point of water (Figs. 2, 3 and 4). Each of the curves if extended to lower and higher salinities would probably assume a more or less sigmoid shape (Jones, 1941; Verwey, 1957), but in other crustaceans previously studied a considerable portion of the curve in the salinity range treated here, 10‰–30‰, is essentially a straight line. In this investigation a linear relationship for salt concentration of blood in this range of salinities is assumed.

Regression coefficients were subjected to analyses of variance and all except one were found significant at the 5% level. The one exception (*duorarum* experimental and control at 28.4° C.) which falls slightly short of this level of significance is attributed to sampling difficulty. If all regression coefficients are considered significant, an important question remains as to whether or not the lines in each figure

TABLE I

The number of determinations and their average values for the blood melting points shown in Figures 2, 3 and 4

		Mean salinity in ‰				
		10.06	15.11	20.17	25.09	30.50
P. aztecus 42–100 mm.						
Exp. & Contr. 28.3° C.	N Determinations	21	23	22	22	23
	$\bar{x}$ Melting point ° C.	−1.21	−1.33	−1.48	−1.56	−1.52
Exp. 48 Hrs. 16.2° C.	N	17	20	20	20	18
	$\bar{x}$	−1.10	−1.29	−1.34	−1.46	−1.57
Contr. 48 Hrs. 28.6° C.	N	4	3	4	4	4
	$\bar{x}$	−1.04	−1.26	−1.45	−1.56	−1.52
Exp. 96 Hrs. 8.8° C.	N	8	18	19	19	20
	$\bar{x}$	−1.13	−1.29	−1.41	−1.53	−1.75
Contr. 96 Hrs. 28.8° C.	N	5	6	6	5	6
	$\bar{x}$	−1.23	−1.33	−1.46	−1.45	−1.56
P. aztecus 120–150 mm.						
Exp. & Contr. 28.3° C.	N	10	10	9	9	8
	$\bar{x}$	−1.36	−1.44	−1.57	−1.69	−1.76
Exp. 48 Hrs. 16.2° C.	N	5	7	8	8	8
	$\bar{x}$	−1.22	−1.26	−1.41	−1.48	−1.58
Exp. 96 Hrs. 8.8° C.	N	2	4	7	7	8
	$\bar{x}$	−0.99	−1.24	−1.34	−1.56	−1.77
P. duorarum 35–100 mm.						
Exp. & Contr. 28.4° C.	N	24	26	26	26	26
	$\bar{x}$	−1.48	−1.45	−1.47	−1.54	−1.65
Exp. 48 Hrs. 17.8° C.	N	22	24	23	24	21
	$\bar{x}$	−1.32	−1.35	−1.47	−1.59	−1.65
Contr. 48 Hrs. 28.4° C.	N	6	6	5	6	6
	$\bar{x}$	−1.45	−1.51	−1.49	−1.62	−1.65
Exp. 96 Hrs. 8.75° C.	N	24	24	24	23	24
	$\bar{x}$	−1.27	−1.32	−1.45	−1.64	−1.74
Contr. 96 Hrs. 28.1° C.	N	6	6	6	6	6
	$\bar{x}$	−1.45	−1.50	−1.49	−1.62	−1.65

are essentially alike, and analyses of covariance (Snedecor, 1956) reported below were used to facilitate these comparisons.

There are at least three confounding factors in the preceding methods. The first two, non-uniform samples and variable warming rate of the brine solution, have been mentioned but the third requires some discussion. The source of dilutant was tap water rather than distilled water because the supply of distilled water was limited. This choice was made deliberately, in full knowledge that the tap water was hard. Salinities of the mixtures were determined by hydrometer, but because many mixtures were measured and averaged in determining the results, the readings were very close to titration values. As an added check, however, samples of the mixtures were taken in capillary tubes and determined by melting point methods, and here departure from expected values became apparent. The greater the dilution the more the melting point was depressed.

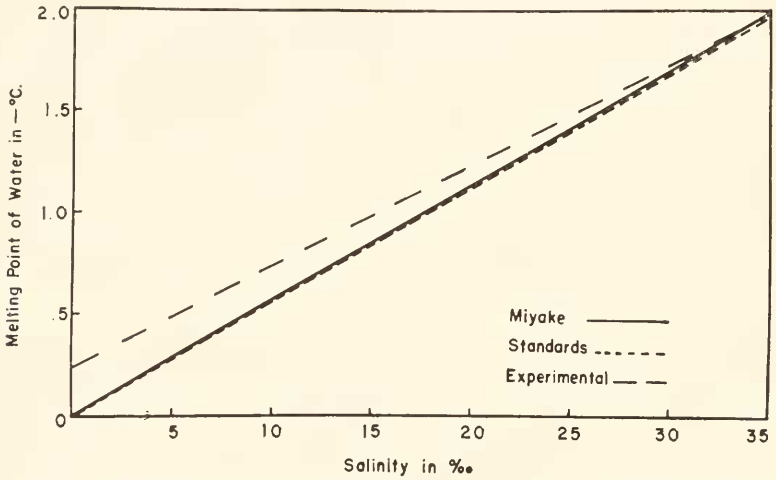

FIGURE 1. Comparison of the relationship between salinity and freezing point depression found by Miyake, that obtained for the standards used in these experiments, and that found in the experimental mixtures of tap water and sea water at average hydrometer-established salinities of 10.06, 15.11, 20.17, 25.09 and 30.50‰.

To determine exactly where the disparity in results lay, the values of Miyake (1939) were used as a basis for reference (Fig. 1). Next to the line for Miyake's values for salinity of west Pacific waters is a line formed by standards mixed for the series of experiments as determined by the differential thermometer. For all practical purposes the lines are identical. A line drawn through mean melting-point values for mixed salinities, however, does not coincide with the first two lines and indeed the deviation appears to be directly proportional to the amount of tap water used in dilution. This disparity is not deemed great and the melting points on the abscissa of Figures 2, 3 and 4 are those determined experimentally and shown in Figure 1. The effect of tap water on osmoregulatory behavior of the shrimp remains unknown.

Results

Juvenile and subadult *aztecus* and *duorarum* are hypotonic to sea water in a salinity range of 30–34‰ in summer in a temperature range of 23–31° C. This was shown by a comparison of the melting points of blood from shrimps and water taken from outside holding tanks at the inception of each weekly experiment. This finding is in accord with the account for a number of decapod crustaceans (*supra cit.*).

Results of six weekly experiments on *aztecus* ranging in total length from 42–100 mm. are shown in Figure 2. Though shrimp in high salinity water prior to experiment are hypotonic, they are hypertonic to hypotonic in the array 10–30‰ after 2–3 hours of immersion in the mixtures at a mean room temperature of 28.3° C., those in the low salinities having more dilute blood than those in high salinities, for the line is not horizontal (b = .347). At the end of 48 hours, with an accompanying drop in temperature to 16.2° C., essentially the same relationship exists though blood in low salinities is even more dilute (b = .464). Controls at room temperature 28.6° C. show a somewhat similar picture (b = .512). At the end of 96 hours

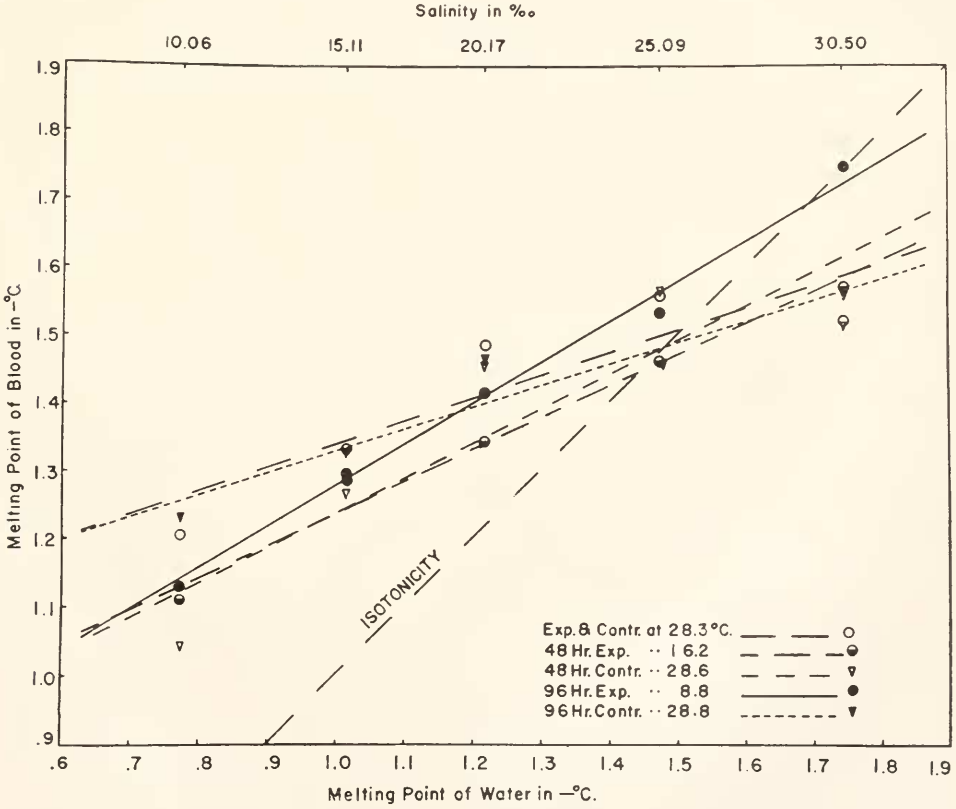

FIGURE 2. *Penaeus aztecus*, 42–100 mm. total length. Regression lines drawn through average melting points of blood at average melting points of water of −0.77, −1.01, −1.22, −1.47 and −1.74° C. Key to lines on figure.

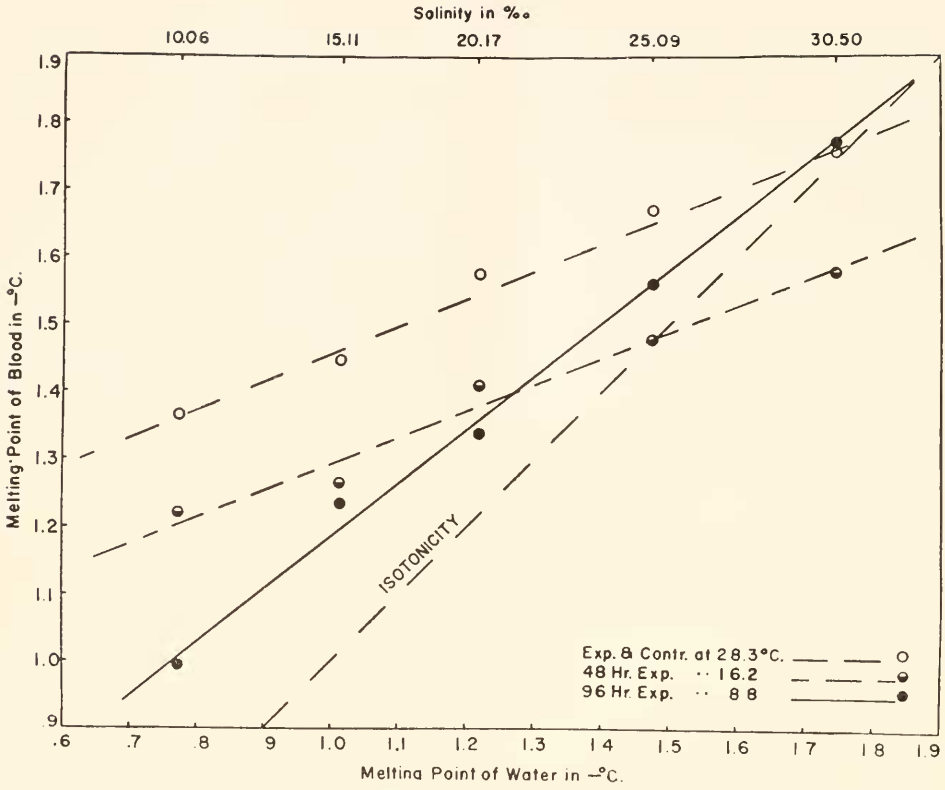

FIGURE 3. *Penaeus aztecus*, 120–150 mm. total length. Regression lines drawn through average melting points of blood at average melting points of water of —0.77, —1.01, —1.22, —1.47 and —1.74° C. Key to lines on figure.

at 8.8° C. the slope of the line has changed markedly (b = .613), the low values are nearly the same as those at 48 hours but values in the upper salinities have raised. The slope of the line indicates a trend toward isotonicity. At the same time the controls in room temperature, 28.8° C., have returned essentially to the same point at which they started (b = .323).

Analyses of covariance of data for initial experimental and control animals paired with 48-hour controls, 96-hour controls and experimental animals at 16.2° C. show no significant difference in slope or elevation ($P = 0.05$), but the line for animals in 8.8° C. water differs significantly in slope.

In larger *aztecus* (Fig. 3), essentially the same circumstances prevail though in accented form. The animals hypotonic to the sea water from which they were taken show a quick response to lowered salinities at room temperature (b = .427). At 48 hours with temperature lowered to 16.2° C. the slope of the line is approximately the same as in the beginning but the concentration of all bloods has been lowered (b = .385). At 96 hours the approach of the slope of blood salinities to the line

of isotonicity is even more marked than in the experiments with younger *aztecus* (b = .783).

These lines represent averages from only two 96-hour runs; hence less reliance can be placed on them than those of the first experiment and, moreover, there are no controls with which to compare the results. The experiments were done in the hotter weeks of summer when aeration of aquaria was essential, especially when the jars held large animals. During one of the experiments the air-pump for controls stopped and during the other heat was above 30° C. In both cases distress and death among controls was too great to give valid results.

Analyses of covariance of the data for initial experimental and control animals paired with experimental animals at 16.2° C. shows that the slopes are parallel but the elevations differ significantly, and the line for animals in 8.8° C. water differs significantly in slope ($P = 0.05$).

Results of six weekly experiments on *duorarum,* 35–100 mm. total length, are shown as a series of regression lines in Figure 4. Again, animals hypotonic to the external medium at ambient temperature of 28.4° C. quickly make an adjustment

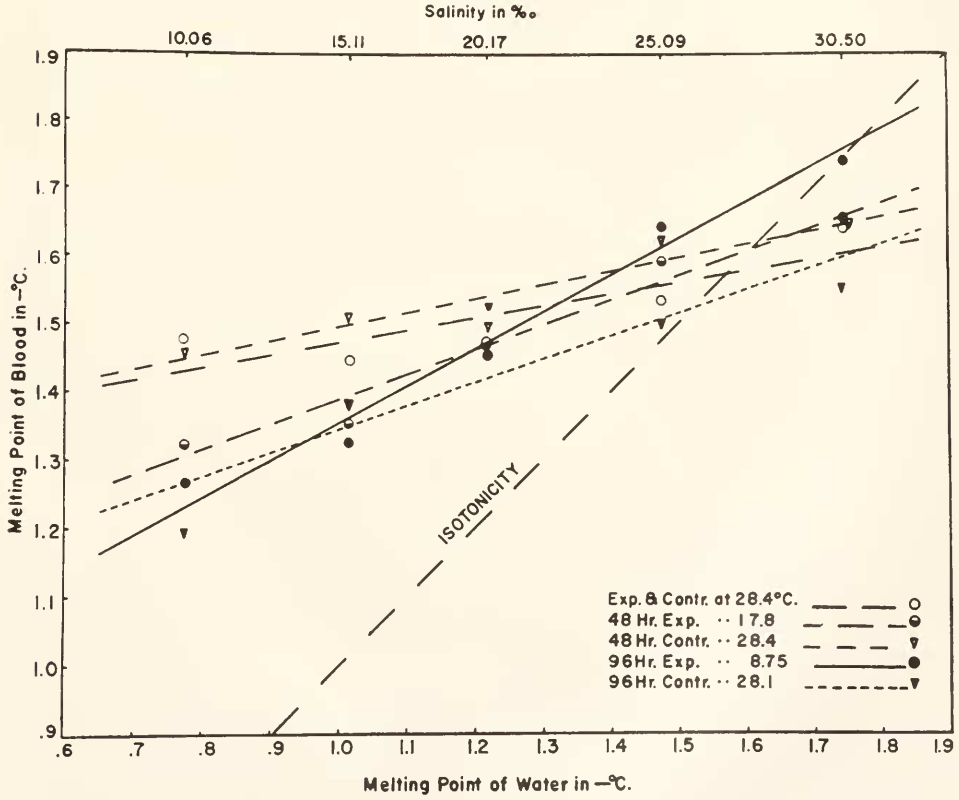

FIGURE 4. *Penaeus duorarum,* 35–100 mm. total length. Regression lines drawn through average melting points of blood at average melting points of water of —0.77, —1.01, —1.22, —1.47 and —1.74° C. Key to lines on figure.

when immersed in an array of 10–30‰ sea water mixtures (b = .182). Within three hours those in dilute media have more dilute blood than those in the less dilute. At the 48-hour point, with temperature gradually depressed to 17.8° C., the slope of this line has changed materially (b = .376). Though animals in 20‰ water have changed little, those in lower salinities have more dilute blood than in the beginning and those in high concentrations have more concentrated blood. Controls at 28.4° C. are essentially unchanged from conditions at the start, but those in higher salinities have somewhat more concentrated blood (b = .210). By the end of 96 hours at a temperature of 8.75° C. a marked change in the slope of the line for blood concentrations is again seen, with a trend toward isotonicity (b = .525). This trend is not so pronounced as in *aztecus* of comparable size (Fig. 2). In controls at 28.1° C. there has been a material dilution of blood at lower salinities (b = .343).

TABLE II

Per cent survival of shrimp at 48 and 96 hours. Forty-eight and 96 hours indicated as mean temperatures for those points. Significant figures in columns for Chi-squares are marked with an asterisk

				Per cent survival						
				Salinity					χ_a^2	χ_b^2
				10 ‰	15 ‰	20 ‰	25 ‰	30 ‰		
P. aztecus 42–100 mm.	Exp.	48 Hrs.	16.2° C.	58.8	83.6	90.0	92.5	88.8	17.98*	4.37
		96	8.8°	37.5	77.5	85.0	87.5	80.0	41.55*	10.30*
	Contr.	48	28.6°	70.8	79.2	95.8	91.7	100.0	3.29	1.25
		96	28.8°	45.8	66.6	70.8	83.3	95.8	12.46*	5.42
P. aztecus 120–150 mm.	Exp.	48	16.2°	28.6	64.3	92.9	96.4	96.4	18.08*	3.79
		96	8.8°	7.1	46.4	89.3	78.6	92.9	38.11*	13.97*
P. duorarum 35–100 mm.	Exp.	48	17.8°	67.7	92.5	98.9	98.9	97.8	10.27*	0.59
		96	8.75°	63.4	89.2	97.8	94.6	96.7	13.82*	1.39
	Contr.	48	28.4°	66.7	86.4	91.7	100.0	100.0	3.88	1.21
		96	28.1°	62.5	77.3	91.7	95.8	100.0	5.63	2.25

Analyses of covariance of the data for initial experimental and control animals paired with 48-hour controls and 96-hour controls show no significant difference in slope or elevation ($P = 0.05$); however, the lines representing data for experimental animals at 17.8° C. and 8.75° C. both differ significantly in slope from the line representing the initial animals ($P = 0.05$).

It is instructive to compare survival of shrimp in the array of salinities and temperatures with the results of melting point determinations as expressed in regression lines (Table II, Figs. 2, 3, 4). Some deaths were due to injury, but if we examine the percentages (Table II) it is apparent that poorest survival was in 10‰ salinity. Chi-square (χ_a^2) values for differences in survival among salinities are significant in seven of the ten series ($P = 0.05$), indicating a high influence of salinity on survival. However, if we eliminate the animals so obviously affected by

10‰ salinity from the Chi-square totals (χ_b^2), it is apparent that higher salinities have little effect on survival. The only significant (χ_b^2) values ($P = 0.05$) are for *aztecus* young and adults at 96 hours in 8.8° C. water. This indicates a response to temperature such as suggested by the regression lines in Figures 2 and 3. Further, it is apparent that survival of *duorarum* is on the whole better than young *aztecus,* indicating greater tolerance to lowered salinity and temperature.

Discussion

Melting point determinations show a high degree of individual variation, as has been noted by Gross (1957b) for *Pachygrapsus crassipes.* Such variations may be attributed to stage in the molting cycle, age, sex, and in the present case perhaps to changes in salinity and non-uniformity of sample size. Replications tend to smooth these inequalities.

Each regression coefficient, except one, is significant at the 5% level. The exception is that for *P. duorarum* experimental and control at 28.4° C. and this instance nearly attains significance at the 5% level. The reason for this exception remains unexplained, though it is thought that the average blood melting point value of − 1.48° C. in water with a melting point of − 0.77° C. is low and perhaps not a good approximation.

Both of these species demonstrate possession of osmoregulatory powers. It is apparent that in the size ranges studied both species are hypotonic to sea water, but that in water under 30‰ they are hypertonic. This is in accord with Verwey (1957), who has suggested that it may be a general rule that Crustacea which regulate their internal environments are hypertonic in water of low salinity and hypotonic in water of high salinity.

Under the experimental conditions imposed, both species maintained themselves fairly well for limited periods in a range of 10–30‰ sea water, but with the lowering of temperature the regulatory powers meet more resistance. In such circumstances there appears to be a species difference. Juveniles and subadults of *P. aztecus* demonstrate a loss of osmoregulatory ability with lowering temperature which is statistically demonstrable after 96 hours, though a trend toward lowered activity is apparent even at 48 hours. In *duorarum* a significant difference was manifest in both 48- and 96-hour experimentally cooled samples. From the standpoint of per cent survival, though, indications are that *P. aztecus,* normally only a summer resident in North Carolina, does not regulate in lowered salinities at lowered temperatures as well as does *P. duorarum,* which is normally resident in North Carolina in winter in the juvenile state. The results provide experimental confirmation of information gained from field observations.

The results do not corroborate those of Broekema (1941) that salinity of blood increases with a fall in temperature, with the exception of shrimp in the 30‰ water, but it is possible that longer exposure to lowered temperature in a constant salinity might give different results. Likewise, the results are not in accord with those of Verwey (1957) that though an animal may not maintain a constant internal environment, it does tend to maintain a constant difference between internal and external environment in terms of osmotic pressure expressed in atmospheres at different

temperatures. Again, longer exposure to a given temperature might alter this picture.

In both species it is shown that in all salinities the blood tends to approach isotonicity with the surrounding medium as temperature is lowered.

Summary

1. Melting point determinations on blood of two species of shrimp, *Penaeus aztecus* and *P. duorarum*, were made in an array of salinities of 10.06, 15.11, 20.17, 25.09, and 30.50‰ at 28.1–28.8, 16.2–17.8 and 8.75–8.8° C.

2. These shrimp are hypotonic to sea water at room temperature and hypertonic to dilutions of sea water below 30‰.

3. These shrimp regulate moderately well in experimental dilutions at room temperature, though the blood is diluted somewhat in lowered salinities.

4. At lowered temperatures, 8.75–8.8° C., regulatory ability is impaired and blood tends toward isotonicity.

5. *P. duorarum* is a better regulator at low temperatures than *P. aztecus*.

6. Survival of these shrimp is better in higher salinities at low temperatures.

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